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Nutrient recovery from wastewater streams by microalgae: Status and prospects

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ABSTRACT

Disposal of wastewater often results in high nutrient loading into aquatic environments, which may lead to favorable conditions for undesirable phytoplankton blooms. Microalgae are efficient in removing nitrogen, phosphorus, and toxic metals from wastewater under controlled environments. If key nutrients in the wastewater stream can be used to grow microalgae for biofuel production, the nutrients can be removed, thus significantly reducing the risk of harmful phytoplankton overgrowth. This review paper summarizes the major nutrient components of different wastewater streams, the mechanisms of algal nutrient uptake, nutrient removal performance of various species of microalgae when cultured in wastewater, and current microalgae production systems. Finally, new algae cultivation technologies applicable for biofuel production and nutrient recovery in polluted water bodies are discussed.

Contents

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1. Introduction

Large amounts of water used for agricultural, municipal, and industrial purposes result in issues due to the large volumes of wastewater generated. Excessive nutrients in the wastewater, such as nitrogen and phosphorus, may cause eutrophication in lakes and upset the balance of the ecosystem. Eutrophication is a serious environmental problem and has become more widespread since the mid-20th century [1]. According to a survey conducted by the International Lake Environment Committee, 48% of lakes and reservoirs in North America are eutrophic; in Asia and the Pacific, 54%; in Europe, 53%; in South America, 41%; and in Africa, 28% [2]. The adverse ecological impacts caused by eutrophication can be categorized based on three aspects: (1) reduction of biodiversity and replacement of dominant species, (2) increased water toxicity, and (3) increased turbidity of the water and decreased lifespan of the lakes. A recent report shows that the economic loss due to eutrophication in U.S. freshwaters was estimated to be $2.2 billion per year [3]. One specific example of freshwater eutrophication is Ohio's largest inland lake, Grand Lake St. Marys (GLSM). Animal manure applied on cropland in the lake's watershed is one of the primary causes for nutrient overloading, while wastewater treatment plants surrounding the lake contribute 5–10% of the phosphorus load [4]. Algae blooms in GLSM generate health concerns for surrounding residents and directly affect the fishing and tourism industries which play a significant role in the local economy. These challenges reflect the urgent need for an economical approach for reducing the impacts of nutrients in wastewaters.

A major requirement in wastewater treatment is the removal of nutrients and toxic metals to acceptable limits prior to discharge and reuse. However, most conventional wastewater treatment technologies are based on chemical and physical methods that are not economical for the treatment of agricultural wastewater. Microalgae have been proven to be efficient in removing nitrogen, phosphorus, and toxic metals from a wide variety of wastewaters [5–7]. There are extensive studies of algae growth in municipal [8,9], agricultural [10,11], and industrial wastewaters [12,13]. Substantial amounts of nutrient removal and algae biomass production were obtained in these studies. Hence, controlled microalgae cultivation shows promise as a potential biological treatment method for wastewater.

Microalgae, which have the potential to be an environmentally friendly biofuel feedstock, have attracted increasing interest for commercial production. Compared to other biofuel feedstocks, microalgae has the following advantages: (1) microalgae do not compete with crops for arable land and freshwater because they can be cultivated in brackish water and on non-arable land; (2) microalgae can grow rapidly and have high oil contents of 20–50% on a dry weight basis [14]; (3) microalgae have the ability to fix carbon dioxide, thus reducing greenhouse gas emissions and improving air quality; (4) microalgae can utilize nutrients from most wastewaters, providing an alternative method for wastewater treatment; and (5) byproducts of microalgae cultivation after lipid extraction, namely algae biomass residue, can be used as a nitrogen source, such as a protein-rich animal feed or fertilizer for crops [15]. In summary, microalgae cultivation has multiple applications, combining biofuel production, carbon dioxide mitigation, and wastewater treatment.

The cultivation of microalgae for biofuel production has been conducted at both laboratory and commercial scales. Tremendous efforts have been invested in algae strain selection and development of efficient cultivation systems [16,17]. The capital cost of a current algae biofuel production system depends on the design of subsystems, including algae cultivation, harvesting, and lipid extraction. Most biofuel companies have chosen to build large algae cultivation operations along coastal areas in order to utilize seawater as the major water source, thus reducing the cost of water usage [18].

The harvesting of microalgae typically employs methods such as filtration, sedimentation, centrifugation, or flocculation, which can be technically and economically challenging when considering larger production scales. Macroalgae are multicellular and can be more easily harvested, either manually or mechanically, which may suggest that macroalgae is a better candidate for nutrient removal from aquatic environments. However, microalgae usually have higher lipid productivity per cultivation area and, as a result, a greater potential for liquid fuel production (Table 1). As macroalgae generally do not contain lipids and have high carbohydrate contents, they are more favored for the production of biogas or alcohol-based fuels.

The uncontrolled growth of algae blooms as a consequence of eutrophication creates many challenges. While it is imperative to inhibit harmful algae blooms in eutrophic waters, the challenge is to develop efficient methods that remove excessive nutrients from these waters. It is possible to import lipid-rich microalgae species into eutrophic waters for the removal of nitrogen and phosphorus, while simultaneously producing biomass for biofuels. However, the design of cost effective algae cultivation systems in natural waters is the major challenge for this approach. This review paper summarizes

Table 1
Comparison between typical microalgae and macroalgae species.

Category	Representative species	Composition (%w/w)			Lipid productivity ($g\ m^{-2}\ d^{-1}$)	Cultivation methods	Harvesting methods	Reference(s)
		Carbohydrates	Protein	Lipids				
Microalgae	*Scenedesmus obliquus*	10–17	50–56	12–14	2.4–13.5	Open ponds, photobioreactors	Filtration, sedimentation, centrifugation, flocculation	[70,71]
	Chlorella sp.	12–17	51–58	14–22	1.6–16.5			
	Euglena gracilis	14–18	39–61	22–38	7.7			
Macroalgae	*Laminaria* sp. (brown seaweed)	60	12	2	0.7–0.9	Natural stocks, aquaculture	Manual, mechanization	[70,72]
	Ulva sp. (green seaweed)	23–78	10–33	0–6	0.6			

the major nutrient components of different wastewater streams, compares nutrient removal ability and lipid production of some algae species that have been cultured in different sources of wastewater, examines current algae production systems, and identifies the algae cultivation technologies applicable for biofuel production and nutrient recovery in polluted waters.

2. Nutrients from wastewater streams

In the past few decades, tremendous efforts have been put into research of microalgae cultivation in wastewaters. Studies showed positive results regarding the potential of utilizing microalgae to remove nitrogen, phosphorus, and other elements from wastewaters. According to the 2008 Clean Watersheds Needs Survey, the amount of wastewater generated in the U.S. is 12 million t d^{-1} [19]. The compositions of wastewaters vary with sources. The nutrient components greatly affect the growth of microalgae and their lipid content and production. In this section, the nutrient components of different wastewater streams (municipal, agricultural, and industrial) are discussed. Anaerobic digestion effluent, as a special waste stream, is also discussed.

2.1. Municipal wastewater

The increasing urbanization and expansion of urban populations has resulted in greater quantities of municipal wastewater. A city with a population of 500,000 and water consumption of 0.2 t d^{-1} $capita^{-1}$ would produce approximately 85,000 t d^{-1} of wastewater [20]. Table 2 shows the levels of the nitrogen and phosphorus in different wastewaters. Compared with animal wastewater, municipal wastewater has less nitrogen and phosphorus. However, there are often considerable amounts of heavy metals such as lead, zinc, and copper in raw municipal sewage. The traditional municipal wastewater treatment process includes three stages: primary, secondary, and advanced. Buoyant and non-buoyant materials are separated in primary treatment using physical or chemical methods. Dissolved organics and colloidal materials are removed during secondary treatment by biological or chemical treatments. The removal of dissolved inorganic components, including nitrogen and phosphorus, takes place in the advanced treatment process through a number of different unit operations including ponds, post-aeration, filtration, carbon adsorption, and membrane separation. Microalgae cultivation for nitrogen and phosphorus removal has been most extensively studied in municipal wastewaters [8,9,21,22].

2.2. Agricultural wastewater

Agricultural wastewater, which is mainly produced from livestock production, is another major source of wastewater. Current animal feeding operations in the U.S. annually generate more than 450 million t of manure and manure-contaminated runoff water [23]. Livestock operations have shifted from small and medium scale to large scale during the past decade. As a result, nutrients have become spatially concentrated in high livestock production areas [23]. The wastewater produced from animal farms is often rich in nitrogen and phosphorus as shown in Table 2. Approximately half of the nitrogen in animal waste is in the form of ammonium, and half is in the form of organic nitrogen. Factors such as animal diet, age, usage, productivity, management, and location will significantly affect the nutrient content in animal wastewater. The nitrogen-to-phosphorus (N/P) ratio is typically 2–8 for dairy, swine, and beef feedlot wastewater. The traditional treatment of animal manure is land application as fertilizer. However, the nutrients in manure cannot be completely remediated by crops due to different N/P ratio requirements and nutrient availability. Excess nutrients accumulate in the soil, which can increase nutrient losses through runoff, resulting in eutrophication in receiving waters. Other than manure, agricultural runoff can also contain herbicides, fungicides, insecticides, and nitrate and phosphate components from agricultural operations. Agricultural runoff may bring particles, dissolved ions and molecules, and living microorganisms into receiving waters, significantly reducing water quality.

2.3. Industrial wastewater

It was reported that an annual average of 665 billion t of water was used by industries around the world between 1987 and 2003. The total water usage of the U.S. was reported to be approximately a third of the world total [24]. Although it varies depending on the source operations, most industrial wastewaters contain more heavy metal pollutants and less nitrogen or phosphorus than other types of wastewater [25]. Selection of microalgae strains with high metal sorption capacity is crucial to achieve high metal removal efficiency. So far, only a few algal species have been studied for metal sorption ability. There are several reports evaluating the nitrogen, phosphorus and heavy metal removal from industrial wastewater as an algae growth medium, such as those from the carpet industry [12].

Table 2
Total nitrogen (TN) and total phosphorus (TP) content of different waste streams.

Wastewater category	Description	TN (mg L^{-1})	TP (mg L^{-1})	N/P	Reference(s)
Municipal wastewater	Sewage	15–90	5–20	3.3	[73]
Animal wastewater	Dairy	185–2636	30–727	3.6–7.2	[74,75]
	Poultry	802–1825	50–446	4–16	[75,76]
	Swine	1110[a]–3213	310–987	3.0–7.8	[75,77]
	Beef feedlot	63–4165	14–1195	2.0–4.5	[75,76]
Industrial wastewater	Textile	21–57[a]	1.0–9.7[b]	2.0–4.1	[12,78]
	Winery	110[a]	52	2.1	[79]
	Tannery	273[a]	21[b]	13.0	[80]
	Paper mill	1.1–10.9	0.6–5.8	3.0–4.3	[81]
	Olive mill	532	182	2.9	[82]
Anaerobic digestion effluent	Dairy manure	125–3456	18–250	7.0–13.8	[27,83]
	Poultry manure	1380–1580	370–382	3.6–4.3	[84,85]
	Sewage sludge	427–467	134–321	–	[86]
	Food waste and dairy manure	1640–1885[a]	296–302	–	[87]

[a] Total Kjeldahl nitrogen (TKN).
[b] Total orthophosphates (PO_4^{3-}–P).

2.4. Anaerobic digestion effluent

Anaerobic digestion (AD) is a mature technology which uses microorganisms to decompose organic waste and produce biogas. Many AD systems have been built in European countries and the U.S. for municipal, industrial, and agricultural waste treatment. Efforts have been focused on the optimization of biogas yield and degradation of the volatile solids [26]; one neglected aspect is the post-treatment of AD effluent. Most of the AD effluent is separated by a dewatering system into liquid and solid fractions. The solid portion is usually composted then marketed as potting media or soil amendment, while the liquid portion is traditionally used as fertilizer for land application [26]. Excessive land application of AD effluent can result in nitrogen and phosphorus runoff, and may contribute to eutrophication. Efficient and cost-effective nutrient recovery methods should be considered in order to reduce the risk of nitrogen and phosphorus pollution from AD.

Compared with typical agricultural, municipal, and industrial wastewater, AD effluent has relatively lower carbon levels because microbial activity during the digestion converts the carbon to methane [27]. The nitrogen in AD effluent is mainly in the form of ammonium [28]. Dilution of AD effluent is usually needed before feeding to algae in order to avoid the potential inhibition of algal growth due to high ammonium concentration and turbidity [29]. In addition, as there is a significant amount of bacteria in AD effluent, proper pretreatment, such as filtration and autoclave, may be necessary to prevent the contamination of algae production systems [27].

3. Nutrient removal and lipid production by microalgae

3.1. Mechanisms of nutrient removal

In order to maximize nutrient removal from the aforementioned wastewater streams by various species of microalgae, the mechanisms of algal nutrient uptake should be thoroughly understood. The various metabolic pathways of the algal cell can be coarsely categorized by elemental constituent. In addition to the four basic elements, i.e., carbon, nitrogen, phosphorus, and sulfur, ionic components such as sodium, potassium, iron, magnesium, calcium, and trace elements must also be provided for algal growth. Emphasis is usually put on nitrogen and phosphorus. Anthropogenic waste production can significantly increase these nutrients, leading to increased risk for nutrient runoff that can stimulate eutrophication.

3.1.1. Carbon

Carbon, in the form of carbon dioxide, may be fixed from the atmosphere and industrial exhaust gases through the photosynthetic activity of autotrophic microalgae. Some microalgae display heterotrophic behavior, using organic forms of carbon, while others may possess both autotrophic and heterotrophic traits, simultaneously. Carbon can be also utilized in the form of soluble carbonates for cell growth, either by direct uptake or conversion of carbonate to free carbon dioxide through carboanhydrase activity. The use of algae to mitigate carbon dioxide from flue gases is another research focus and, if effective, could benefit both the environment and biofuel production.

3.1.2. Nitrogen

Nitrogen is a critical nutrient required in the growth of all organisms. Organic nitrogen is found in a variety of biological substances, such as peptides, proteins, enzymes, chlorophylls, energy transfer molecules (ADP, ATP), and genetic materials (RNA, DNA) [30]. Organic nitrogen is derived from inorganic sources including nitrate (NO_3^-), nitrite (NO_2^-), nitric acid (HNO_3), ammonium (NH_4^+), ammonia (NH_3), and nitrogen gas (N_2). Microalgae play a key role in converting inorganic nitrogen to its organic form through a process called assimilation. In addition, cyanobacteria are capable of converting atmospheric nitrogen into ammonia by means of fixation.

$$NO_3^- \xrightarrow[\text{NADH} + H^+ \;\to\; \text{NAD}^+ + H_2O]{\text{nitrate reductase}} NO_2^- \xrightarrow[6\text{Fd}_{red} + 8H^+ \;\to\; 6\text{Fd}_{ox} + 2H_2O]{\text{nitrite reductase}} NH_4^+ \xrightarrow[\text{ATP} + \text{Glu} \;\to\; \text{ADP} + PO_4^{3-}]{\text{glutamine synthetase}} \text{L-glutamine}$$

Fig. 1. Simplified schematic of the assimilation of inorganic nitrogen.

Assimilation, which is performed by all eukaryotic algae, requires inorganic nitrogen be only in the forms of nitrate, nitrite, and ammonium. As shown in Fig. 1, translocation of the inorganic nitrogen occurs across the plasma membrane, followed by the reduction of oxidized nitrogen and the incorporation of ammonium into amino acids. Nitrate and nitrite undergo reduction with the assistance of nitrate reductase and nitrite reductase, respectively. Nitrate reductase uses the reduced form of nicotinamide adenine dinucleotide (NADH) to transfer two electrons, resulting in the conversion of nitrate into nitrite. Nitrite is reduced to ammonium by nitrite reductase and ferredoxin (Fd), transferring a total of six electrons in the reaction. Thus, all forms of inorganic nitrogen are ultimately reduced to ammonium prior to being incorporated into amino acids within the intracellular fluid. Finally, using glutamate (Glu) and adenosine triphosphate (ATP), glutamine synthase facilitates the incorporation ammonium into the amino acid glutamine.

Ammonium is thought to be the preferred form of nitrogen because a redox reaction is not involved in its assimilation; thus, it requires less energy. Studies have shown that, in general, algae tend to prefer ammonium over nitrate, and nitrate consumption does not occur until the ammonium is almost completely consumed [31]. Therefore, wastewaters with high ammonium concentrations can be effectively used to rapidly grow microalgae. Although ammonium is preferred by algae, nitrate is the more highly oxidized form and the most thermodynamically stable in oxidized aquatic environments, and thus is predominant [30]. However, nitrate can also be an essential nitrogen source for microalgae as the presence of nitrate induces the activity of nitrate reductase. In contrast, excess ammonium can have a repressive effect [32]. The ammonium tolerance of different algae species varies from 25 μmol NH_4^+–N L^{-1} to 1000 μmol NH_4^+–N L^{-1} [33].One approach to growing microalgae in high ammonium concentrations is utilizing the plant enzyme glutamine synthetase, which has a high affinity for ammonium. The addition of glutamic acid was reported to result in 70% more ammonium reduction per cell during the growth of *Chlorella vulgaris* in natural wastewater [34].

Ammonium is not only removed by cell metabolism, but also by ammonia stripping, where significant amounts of ammonia can be volatilized at increased pH and temperature. Garcia et al. [35] showed that ammonia stripping was the most important mechanism in high growth rate algal ponds operating at various hydraulic retention times. It was also reported that when high rate algal ponds were exposed to warm weather, ammonia release accelerated even when the pH was below 9.

3.1.3. Phosphorus

Phosphorus is also a key factor in the energy metabolism of algae and is found in nucleic acids, lipids, proteins, and the

intermediates of carbohydrate metabolism. Inorganic phosphates play a significant role in algae cell growth and metabolism. During algae metabolism, phosphorus, preferably in the forms of $H_2PO_4^-$ and HPO_4^{2-}, is incorporated into organic compounds through phosphorylation, much of which involves the generation of ATP from adenosine diphosphate (ADP), accompanied by a form of energy input [36]. Energy input can come from the oxidation of respiratory substrates, the electron transport system of the mitochondria, or in the case of photosynthesis, from light. Phosphates are transferred by energized transport across the plasma membrane of the algal cell. Not only are inorganic forms of phosphorus utilized by microalgae, but some varieties of algae are able to use the phosphorus found in organic esters for growth [37].

Although orthophosphate is generally recognized as the limiting nutrient in freshwater systems, many cases of eutrophication are triggered by superfluous phosphorus, which can result from runoff of wastewater [38]. Similar to the removal of nitrogen, it should be noted that phosphorus removal in wastewater is not only governed by the uptake into the cell, but also by external conditions such as pH and dissolved oxygen. Phosphorus cannot exist in a gaseous state, thus phosphate will precipitate from the medium as a result of elevated pH and high dissolved oxygen concentration.

3.1.4. Other nutrients

Although, nitrogen and phosphorous are the two main nutrients of concern in eutrophication, being limiting factors in most growth scenarios [39], other micronutrients, including silicon and iron, can affect the abundance of phytoplankton communities [40]. However, many of the micronutrients are toxic to most algae species at high concentrations. Some of them also form precipitates with other essential elements and reduce their availability. However, some algae strains are particularly tolerant to heavy metals and their potential to absorb metals has been demonstrated [41].

3.2. Species variation of nutrient removal

3.2.1. Chlorophytes (green algae)

Chlorophytes is one of the largest phyla of microalgae, with a vast array of species and a wide geographical distribution. As shown in Table 3, *Chlorella* sp. has been used in numerous studies and shown to be effective in removing nitrogen and phosphorus from different wastewater streams with a wide range of initial concentrations. Nitrogen and phosphorous removal efficiencies from the growth of *Chlorella* sp. range from 8 to 100%. Studies shown in Table 3 also confirm that *C. vulgaris* has higher nutrient removal efficiencies than that of *Chlorella kessleri* when comparing their performances in artificial media. An exceptionally low nutrient removal was found in the growth of *C. kessleri* in which the microalgae were subjected to artificial wastewater for a relatively small amount of time [42]. In other studies, *Chlorella* spp. nitrogen removal efficiency was 23–100%, while phosphorus removal efficiency was 20–100% [22,27,34,43–46]. In addition, it has been reported that *Chlorella* sp. is tolerant to $NH4_4^+-N$ [27].

In order to ensure the simultaneous utilization of both nitrogen and phosphorus, the N/P ratio should be with in a proper range [47]. The optimal ratio differs among cultures due to strain-varying metabolic pathways. The N/P ratio can be up to 250 for healthy freshwater environments, but in most wastewater streams ratios may be as low as 4–5 [48]. An optimal N/P ratio for *C. vulgaris* was reported to be 7 [49], which is in agreement with the N/P ratio of 7.2 calculated from the Stumm empirical formula for microalgae ($C_{106}H_{263}O_{110}N_{16}P$). These ratios indicate that the removal rate of nitrogen would be faster than that of phosphate, since a larger

Table 3
Nitrogen and phosphorus removal by various genera of microalgae and cyanobacteria in the axenic batch processes of different waste streams.

Category	Genus and species	Waste stream	Process type	Removal time (d)	Total nitrogen (TN)		Total phosphorus (TP)		Reference(s)
					Initial conc. (mg L^{-1})	Removal efficiency (%)	Initial conc. (mg L^{-1})	Removal efficiency (%)	
Chlorophyte	*Chlorella* sp.	Digested manure	Batch	21	100–240	76–83	15–30	63–75	[27]
	C. kessleri	Artificial medium	Batch	3	168	8–19[b]	10–12	8–20[c]	[42]
	C. pyrenoidosa	Industrial wastewater	Fed-batch	5	267	87–89	56	70	[44]
	C. sorokiniana	Municipal wastewater	Batch	10	–	–	22	45–72	[88]
	C. vulgaris	Artificial medium	Batch	1–10	13–410	23–100[a]	5–8	46–94[c]	[45]
	C. vulgaris	Industrial wastewater	Batch	5–9	3–36	30–95[a]	112	20–55	[43]
	C. vulgaris	Municipal wastewater	Batch	2–10	48–1550	55–88	4–42	12–100	[22,34]
	C. reinhardtii	Artificial medium	Batch	10–30	129	42–83[a]	120	13–14[c]	[46]
	Scenedesmus sp.	Artificial medium	Batch	0.2–4.5	14–44	30–100[a,b]	1.4–6.0	30–100[c]	[89]
	S. dimorphus	Industrial wastewater	Batch	9	–	–	112	20–55	[43]
	S. obliquus	Municipal wastewater	Batch	0.2–8	27	79–100[a]	12	47–98	[22,52]
Cyanobacteria	*Arthrospira* sp.	Animal wastewater	Semi-cont.	–	–	84–96[a]	–	72–87[c]	[55]
	A. platensis	Industrial wastewater	Batch	15	2–3	96–100[a]	18–21	87–99[c]	[90]
	Oscillatoria sp.	Municipal wastewater	Continuous	14	498	100	76	100	[91]
Diatom	*P. tricornutum*	Municipal wastewater	Continuous	14	498–835	80–100	76–116	50–100	[91,92]
Haptophyte	*I. galbana*	Artificial medium	Batch	8	377	99	–	–	[93]

[a] Ammonia nitrogen (NH_4^+–N).
[b] Nitrate (NO_3^-–N), Nitrite (NO_2^-–N).
[c] Total orthophosphates (PO_4^{3-}–P).

proportion is required. The faster removal of nitrogen over phosphorus was observed in the growth of *Chlorella pyrenoidosa* in soybean processing wastewater [44]. It was observed that the removed nitrogen was mainly used for algal cell synthesis, whereas 17% of the phosphorus was removed via precipitation rather than by assimilation.

Some species of *Chlorella* are heterotrophic or mixotrophic and can consume organic forms of carbon in addition to inorganic nutrients as part of their metabolic process. This can be an advantage when using wastewater streams containing carbon residues, such as digested dairy manure [27]. Acetate, which is found in some wastewaters, was shown to be effectively consumed during heterotrophic or mixotrophic microalgae cultivation [21]. Anaerobically pretreated soybean processing wastewater was shown to improve the growth of *C. pyrenoidosa* by providing additional acetate and small organic molecules [44]. Heterotrophic growth is not an advantageous strategy in wastewaters deficient in organic carbon. Under heterotrophic conditions, the addition of carbon in the form of sodium acetate or glucose was necessary to achieve ammonium removal at a level equivalent to that under autotrophic conditions for the growth of *C. vulgaris* [50].

Another chlorophyte widely used for nutrient removal studies is *Scenedesmus* sp., a small non-motile green algae, often clustered in colonies consisting of 2, 4, 8, 16 or 32 cells. The cells are equipped with spines and bristles, which make the colonies more buoyant and allow increased light and nutrient uptake while deterring predation in the water. Data in Table 3 show that the nitrogen and phosphorus removal efficiency of *Scenedesmus* spp. was 30–100%. Its nutrient uptake behavior was not significantly different from that of some *Chlorella*, as shown by a comparison of *Scenedesmus dimorphus* and *C. vulgaris* [43]. The study did note, however, that the removal of ammonium by *S. dimporphus* was significantly greater than that of *C. vulgaris* at an incubation time of less than 9 days (220 h). A different study showed that when immobilized in alginate, the ammonium removal efficiency of *Scenedesmus obliquus* was higher than that of *C. vulgaris* [22].

It was reported that *Scenedesmus* sp. requires an N/P ratio of approximately 30 to grow without limitation by either nutrient [51]. When grown in an environment with N/P ratios between 12 and 18, the microalgae had a continuous nitrogen limitation, resulting in a high internal phosphate pool [52]. Thus, the subsequent nitrogen removal rates were always shown to be greater than that of phosphorus. The high N/P ratio requirement could possibly explain the low phosphorus removal of 20–55% from agricultural wastewater by *S. dimorphus* [43].

Other genera of green algae are also capable of effectively removing nutrients from wastewater. Sawayama [53] found that *Botryococcus braunii* grown in treated sewage from municipal wastewater was able to consume nitrate and nitrite, but did not remove ammonium. Ammonium was reported to be inhibitory to cell growth in this particular culture. *Chlamydomonas reinhardtii* was capable of removing 42–55% of ammonium and 13–15% of phosphorus from an artificial medium with an N/P ratio of approximately 1 [46]. The removal efficiency was slightly increased when scaling up the process 45- or 90-fold in a biocoil reactor [46].

Non-axenic cultures, which are a mixture of different species of algae, can also be used to remove nutrients from wastewater. A combination of *C. vulgaris*, *Scenedesmus falcatus*, *Chlamydomonas mirabilis*, and *Microcystis aeruginosa* showed a 58% reduction in ammonium and 34% reduction in phosphates during the algal treatment phase of a city sewage treatment process [54].

3.2.2. Cyanobacteria (blue–green algae)

Cyanobacteria are considered to be the most common and problematic algal taxa found in nuisance blooms. Many species of cyanobacteria can be toxic, posing serious environmental, and health issues. However, some genera such as *Arthrospira* contain a high amount of essential amino acids, vitamins, and fatty acids, and thus can be used as nutritional products or animal feed. Unlike eukaryotic algae, blue–green algae are members of the bacterial domain, but are still extensively researched due to their ability to obtain energy through photosynthesis, assimilate inorganic nutrients, and fix atmospheric nitrogen.

Cyanobacteria *Arthrospira* sp. was shown to be capable of removing 84–96% of ammonium nitrogen and 72–87% of phosphorus from pig wastewater [55]. Ammonium removed at a rate of 13.6 mg L^{-1} d^{-1} corresponded to 93% removal [55]. Nitrate uptake by *Phormidium laminosum* and also *Synechococcus elongatus* was inhibited by ammonia [56]. It was also observed in the same study that *Phormidium laminosum* preferred to assimilate nitrite over nitrate.

The favorable range of N/P ratios varies by species, but it appears that cyanobacteria favor low N/P ratios for maximum nutrient removal. The average phosphate removal rate of *Phormidium bohneri* grown in the secondary effluent of a municipal wastewater treatment plant increased 8.6-fold when the N/P ratio decreased from 6 to 1 [57]. An analysis of 17 different lakes showed that cyanophyta was more dominant than other algal taxa at N/P ratios less than 29 [58]. However, Suttle and Harrison [59] reported that *Synechococcus* sp. was able to outcompete green alga *Scenedesmus* sp. or diatoms *Synedra* and *Nitzschia* when subjected to a high N/P ratio of 45.

3.3. Environmental factors

A number of environmental factors affect the rate of nutrient uptake for various species of microalgae, including initial nutrient concentration, light intensity, extracellular pH, temperature, and inoculation density. Among these parameters, nutrient concentration and light intensity have direct impacts on nutrient removal. Other parameters indirectly affect the nutrient removal through the change of nutrient concentration or light intensity.

The overall composition of the nutrient source affects the nutrient uptake. Nitrogen or phosphorus is often the limiting nutrient in the substrate medium, and thus an optimal N/P ratio exists where the maximum nutrient removal is found [60]. However, the optimal N/P ratio differs by species. In general, the absolute amount of nitrogen or phosphorus was seen to impact the metabolism of algal cells in a similar manner. Most of the reviewed studies proposed a particular initial nutrient concentration range for optimal cell growth and nutrient removal. A number of studies found that the rate of phosphorus removal was proportional to the initial nutrient concentration [51,57]. A kinetic study on *S. obliquus* also found that the specific uptake rate of phosphorus increased with the initial concentration, only to converge to a constant value [36]. However, the uptake rates of ammonium and phosphorus in *C. vulgaris* were observed to drastically decrease at extremely high concentrations, possibly due to the excessive production of chlorophyll and subsequent light limitation due to self-shadowing [45]. It was found that when nitrogen was sufficient, *P. bohneri* was not able to consume phosphorus [57]. For heterotrophic or mixotrophic conditions, an additional carbon source, such as glycerol or glutamic acid, caused an increase in the uptake of nitrogen [34].

The concentration of nutrients can be affected by pH. Drastic changes in pH can change the solubility of ammonium or phosphates present in the substrate medium, and elevated pH levels can result in ammonia stripping or phosphate precipitation. pH values between 9 and 11 induced the precipitation of phosphorus in the form of calcium phosphate [57].

Light intensity is also an important factor affecting nutrient uptake since it supplies energy to the algal cells. The removal efficiency of organic carbon and phosphorus with the heterotrophic *Chlorella kessleri* was reported to be greater under a 12 h light/12 h dark lighting scheme than that under continuous lighting, while nitrate removal efficiency under continuous lighting was greater than that under diurnal illumination [42].

3.4. Lipid production

Although the main focus of this article is to assess the viability of using microalgae for the recycling of inorganic nutrients found in wastewater, it is equally important to consider the utilization of the cultivated biomass after wastewater treatment. Many species of microalgae are known to have high content of lipids, which have the potential to be converted to alternatives to petroleum based diesel or biodiesel from terrestrial plants [14]. The total cost of algal lipid production is directly associated with the lipid production efficiency. The energy consumption per liter (L) of harvesting volume in algal biofuel production was shown to drop from 2600 to 75 $kJ\,L^{-1}$ when the lipid content of algae biomass increased from 2% to 30% [61]. Based on a study of both experimental and commercial scale systems, the cost of algal biofuel production for a highly productive system was found to be mainly related to growth (77%), harvesting (12%), and extraction (7.9%) [61]. It was anticipated that the utilization of wastewater as a substitute for commercial algae nutrients would significantly reduce the operational cost of algae cultivation. The recent focus on maximizing the growth yields and lipid content of microalgae has somewhat shifted the interest from nutrient removal. Thus, there is a significant lack of data for the nutrient removal for genera recognized to have high lipid contents, such as *Nannochloropsis* or *Chlamydomonas.* Nonetheless, some algal species, for which there is sufficient nutrient removal data, have also been found to have high lipid contents when grown in wastewaters. *C. vulgaris* grown in an artificial medium resulted in 20–42% (dry basis) lipids while reporting removal efficiencies of 97% for ammonium and 96% for total phosphorus [62]. *C. pyrenoidosa* was also shown to adapt and utilize high concentrations of organic substrates, resulting in a large growth rate [44].

It should be noted that lipid accumulation drastically increases under nutrient-deprived conditions, and biomass accumulation occurs when nutrients are sufficient [63]. Algae growth systems can be optimized in two-stage systems in which nutrients are effectively depleted from a wastewater source while maximizing cell growth and, subsequently, the lipid content is increased under a nutrient-deprived environment.

4. Microalgae cultivation systems

To realize the commercialization of microalgae-based fuels, a number of needs must be met: (1) inexpensive culture media, (2) low cost algae production systems that can be easily installed and maintained, (3) sufficient carbon dioxide sources for optimal algae growth, (4) efficient algae harvesting methods, and (5) cost-effective and low-energy input algal lipid extraction methods. Growing algae in nutrient-rich wastewaters could help address the first requirement and, when combined with an economical algae cultivation system, could provide a cost effective supply of algae biomass to downstream processing. Most of the current algae cultivation systems can be categorized into three groups according to their reactor design: open systems, closed systems, and hybrid systems, all of which are used on land. The major difference among these systems is whether the algae are exposed to the surrounding environment. However, they also share one common feature, that is, they all utilize suspended cultures in an aquatic environment.

4.1. Suspended culture systems

4.1.1. Open systems

The raceway pond, a typical open system for algae cultivation, has been extensively used since the 1950s. A raceway pond is usually only about 0.3 m deep to provide sufficient sunlight to allow photosynthesis by microalgal cells. The algae culture is mixed and circulated around the raceway track by paddlewheels [14]. Currently, most commercial scale algae cultivation systems are open ponds because they are relatively inexpensive to build and easy to scale up. Some algae ponds are built on non-arable lands adjacent to power plants to have access to carbon dioxide from flue gas. Some are built near wastewater treatment plants to easily access nutrient supplies. During recent years, a significant number of demonstration scale algae ponds have been under construction in California, New Mexico, Hawaii, and Florida. Efforts have been focused on the improvement of algal biomass production through the amplification and optimization of the algae pond systems by industrial companies. Sapphire Energy Inc., headquartered in San Diego, has broken ground on a 121-ha commercial demonstration facility consisting of ten 4-ha ponds in New Mexico, with the goal of producing 16,000 L of fuel per day by 2014 [64]. PetroSun Biofuels Inc. has built saltwater algae ponds on 445 ha on the coast of South Texas near South Padre Island. It is claimed that the ponds will produce 16 million L of algal oil and 49,895 t of biomass per year [65]. Aurora Biofuels is developing wastewater treatment models with algal ponds in Florida and expects to have commercial-scale facilities in 2012.

Despite the economic advantage of open ponds, disadvantages exist in many aspects. First of all, since the system is exposed to the atmosphere, water loss by evaporation increases considerably as the temperature increases. Contamination is another inevitable issue with this system. Starting with large amounts of inoculum and frequent feeding or harvesting could help maintain the dominance of selected algae strains. When it comes to economics, an open pond system may be the first choice for nutrient recovery from wastewater. However, if wastewater is used as a nutrient source for large algae ponds, sterilization may be necessary in order to minimize the negative effects of pathogens and bacteria on algal growth. However, this process also increases the capital cost of the algae cultivation system.

4.1.2. Closed systems

Compared with open ponds, the design of closed reactors helps avoid water evaporation and contamination, and increases photosynthesis efficiency. Typical closed reactors include flat plate reactors, tubular photobioreactors, and bag systems [66]. Flat plate and tubular reactors are well designed to ensure optimal light availability and gas exchange. Sunlight capture can also be maximized by changing the arrangement of the reactor tubes according to the orientation of the sun. Though algal biomass production could be significantly improved, installation and maintenance costs of these reactors are much higher than open ponds, which is the major limiting factor for commercialization of closed systems. Bag systems use large plastic bags approximately 0.5 m in diameter fitted with aeration systems [66]. The maintenance of this type of system is labor intensive and the algae culture usually crashes due to inadequate mixing.

Closed reactors are suitable for photoautotrophic, mixotrophic, or heterotrophic algae. Some algae strains need to be grown free of contaminants in closed reactors for the production of high-value algal products for food and pharmaceutical industries.

Some companies have also been working on the scale up of closed reactors at low cost. The Lumian™ AGS4000 is a 4000-L high productivity algae growth system developed by Solix BioSystems. This system includes a growth basin with 20 Lumian panels integrated with a support system for media preparation, harvesting, reinjection, and system cleaning. This system is able to provide a large amount of pure inoculum for multiple algal ponds. Algenol Biofuels Inc.'s 15-ha facility in Florida contains 3000 of Algenol's patented photobioreactors in a commercial module with a target capacity of approximately 379,000 L of fuel-grade ethanol per year.

4.1.3. Hybrid systems

Hybrid systems combine the merits of open and closed systems in a two-stage cultivation system. The first stage uses closed photobioreactors to culture the inoculum for the second stage where algae are cultivated in open ponds. In this way, cultures are exposed to minimal contamination before being fed to large scale ponds. Sufficient seed supplies from photobioreactors also help keep the preferred algal species dominant in the ponds. However, large-scale applications have been limited by the cost of the first stage. The company Cellana is trying to commercialize its patented algae production system, which couples closed-culture photobioreactors with open seawater ponds in a two-stage process, in Hawaii. The commercial scale facility is to be located adjacent to a power plant to mitigate its carbon dioxide emissions. Green Star Products, Inc. (GSPI) has successfully passed the winter environmental test of its outdoor hybrid algae production facility in Montana. The 40,000-L demonstration facility endured severe Montana winter conditions which included many night-time temperatures below $-18\,^{\circ}C$ and snowfalls of up to 355 mm. The system was maintained with a small generator (under 1 kW demand) to provide electric power for the water pumping system. The carbon dioxide from the exhaust produced by the generator was fed to the algae and the exhaust heat was passed into the algae water, which provides heat for the culture.

4.2. Algae immobilization

The harvest or separation of algae biomass from the culture medium or treated wastewater is one of the major bottlenecks for large algal biofuel production systems because the size of microalgae cells is very small and cultures are usually quite diluted. Current algae harvesting methods include chemical, mechanical, electrical, and biological techniques. The large consumption of chemicals and energy are major challenges and limit the use of these methods. Immobilization of algal cells, in which natural or artificial methods prevent the algae from moving freely within the system, is an alternative method that offers several advantages. Higher removal rates are claimed to be obtained through immobilized algae [67]. In addition, it is easier to control the algae species as washout of the cultivated species is minimized or avoided, and the effluent coming out from the system is cell-free and ready to be processed or reused for other purposes [67].

The main focuses of immobilization technology are the immobilization techniques and the design of the bioreactors. Currently, there are six types of immobilization methods (covalent coupling, affinity immobilization, adsorption, confinement in liquid–liquid emulsion, capture behind semipermeable membrane, and entrapment) and five types of bioreactors (fluidized-bed, packed-bed, parallel-plate, air-lift, and hollow-fiber) [68]. As reported, most algal immobilization research is at laboratory scale and entrapment is the most frequently used method in laboratory experiments [68]. Cells are confined in a three-dimensional gel lattice but are free within their compartments. Interchanges of substrates and products occur through the pores in the material, which can be a synthetic (i.e., acrylamide, polyurethane, polyvinyl) or natural polymer (i.e., collagen, agar, agarose, cellulose, alginate, carrageenan).

Uptake rates for both nitrogen and phosphorus by immobilized algal cells were higher compared with their free-living counterparts. However, the removal of phosphate was much slower than the removal of nitrogen [68]. Although algal immobilization has several advantages over traditional algae cultivation based on nutrient removal rates and downstream processing such as harvesting and water recycling, the effects of immobilization on cell behavior were mostly negative according to observations, especially for growth rate and biomass productivity [67]. High densities of the immobilization matrix as well as a high immobilized cell density per bead was shown to reduce the light penetrating through the system, thus reducing the metabolic activity [22]. High cost is also a limiting factor for wide application of immobilization technologies in wastewater treatment.

4.3. Innovative submersible aquatic algae cultivation technology

Culturing algae in existing nutrient-rich water bodies (wastewater lagoons, fish farms, polluted lakes and reservoirs, and coastal waters) is a new attempt to trap nitrogen and phosphorus from the polluted aquatic systems and produce algal biomass and biofuel at the same time. The floating pond invented by Bussell [69] is one innovative technology that could grow algae in a buoyant system suspended in water. The algae are grown in a controlled space separated from surrounding water, reducing uncontrolled algae blooms throughout the polluted waters. This floating system takes advantage of the natural mixing by wind and waves to achieve high carbon dioxide transfer rates with the atmosphere, thereby reducing the cost of energy input for carbon dioxide addition and mixing devices. Algae species with high nutrient uptake capability can be adopted to maximize the nutrient removal rates from the polluted waters. This system promotes microalgae growth which is beneficial to the improvement of water quality. Energy Dynamics Laboratory is also developing a floating algae pond with low-maintenance requirements. The positive performance of the ponds is highly dependent on the weather conditions including wind, temperature, and sunshine. It is claimed that floating ponds will ideally produce 12,200–14,000 $L\,ha^{-1}\,yr^{-1}$ of biofuel, more than twice of that produced from palm oil (5600 $L\,ha^{-1}\,yr^{-1}$). There is a risk of the controlled microalgae escaping from the enclosed area upon failure of the containment system, and thus cautionary measures should be taken in using this technology.

5. Conclusions

Although the ability of microalgae to assimilate excess nutrients from the environment has been thoroughly studied, due to the complex characteristics of wastewater, the tests of growing algae in wastewater are mostly at laboratory scale. Pilot-scale algae cultivation continues to face many issues including contamination, inconsistent wastewater components, and unstable biomass production. The major challenge associated with culturing algae in nutrient-rich, natural water bodies comes from the design of the cultivation system. Further research is needed to identify algae species and optimize operating parameters for lipid production that can be used to prevent eutrophication of water bodies.

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